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13. ABSTRACT *(Maximum 200 words)*

The multidomain nature of the L protein inferred by sequence similarity was partially confirmed by independent expression of separate protein domains

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DISSECTION OF THE LARGE MULTIFUNCTIONAL L PROTEIN OF VESICULAR STOMATITIS VIRUS: MAPPING FUNCTIONAL DOMAINS

FINAL REPORT

APRIL 9, 2002

U. S. ARMY RESEARCH OFFICE

DAAHO4-95-1-0351

UNIVERSITY OF KENTUCKY

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1. Foreword (Optional): NA

2. Table of Contents (If more than 10 pages): NA

3. List of Appendixes, Illustrations and Tables (If Applicable) NA

4. Body of Report

   A. Statement Of Problem Studied:

      The L protein contains multiple putative domains. The activity of each domain and contribution to overall activity was studied. See appended abstract.

   B. Summary Of The Most Important Results:

      The activities of certain domains were established by separate expression.

   C. List of All Publications and Technical Reports:

      Poster abstracts appended. Manuscripts to be forwarded when available.

   D. List Of All Participating Scientific Personnel Showing Any Advanced Degrees Earned By Them While Employed On The Project*:

      Judith A. Lesnaw
      Amy Gorman
      Michael J. Bass
      Sharon Ray
      Thomas d'Andrea

5. Report of Inventions (By Title Only)* NONE

6. Bibliography: NA

7. Appendixes: NA

*TO BE INCLUDED ONLY IN FINAL REPORT

PROJECT ABSTRACT

The research described in this proposal is focused on mapping functional domains within a large (2,109 amino acids), multifunctional protein that displays six co-coordinate enzymatic activities required for the synthesis of viral mRNA, and a variety of protein-protein, protein-RNA, and protein-substrate interactions. This protein, called "L" to reflect its large size, is encoded in the genome of vesicular stomatitis virus (VSV). VSV stores its genetic information in a single-stranded, nonsegmented RNA genome that is complementary to the viral mRNA. The first biosynthetic event in the life cycle of VSV is the production of mRNA through transcription of the viral RNA genome. The process is directed by a transcription complex (TC) that comprises an RNP template (N protein and genomic RNA) and a transcriptase (L and P proteins), both of viral origin. Six enzymatic activities, RNP-dependent RNA polymerase, guanylyltransferase, guanine-7, and nucleoside 2'-O-methyltransferases, poly A polymerase, and serine/threonine kinase activities, all required for mRNA synthesis, are displayed by the TC. Previous genetic, biochemical, and photoaffinity labeling analyses indicated that the L protein is the multicatalytic element of the TC. The long range goal of this proposal is to correlate the structure of the L protein with its multiple, coordinated functions. These studies will provide useful information for the design of novel multifunctional proteins with useful constellations of catalytic activities. The specific objectives for the requested funding period are focused on identifying functional domains of the L protein through integration of data derived from the following experimental approaches:

I. Nucleotide substrate-binding domains of the L protein will be identified by domain-directed photoaffinity labeling using azido nucleotide analogs (azido ATP, UTP, GTP, and AdoMet). The protein kinase domain will be investigated in year 1, the polymerase and guanylyltransferase domains in year 2, and the two methyltransferase domains in year 3.

II. The hypothesis that the regions of the L protein identified through photolabeling correspond to catalytic domains will be tested, and the functional significance of the regions will be defined by structural and functional analyses of recombinant L proteins (expressed in insect cells) that have been altered at conserved amino acids within the photolabeled domains by site-directed mutagenesis (SDM). These analyses will be initiated as domains are identified.

III. Regions of the L protein that affect catalytic function will be identified through sequence analysis of the L genes of previously isolated ts mutants that display altered polymerase and methlytransferase activities. Analysis of two mutants and their revertants will be initiated in year 1. Multiple amino acid mutations potentially identified will be recreated individually in the wild type clone, and the recombinant L proteins will be expressed in insect cells in year 2, and characterized in year 3.

IV. The hypothesis that highly conserved regions of the L protein, that bear similarity to ATP-binding, RNA-binding, RNA polymerase, and guanylyltransferase motifs, constitute functional domains will be tested by in vitro functional analyses of recombinant L proteins altered by SDM and expressed in insect cells. Mutants will be constructed in year 1, and their functional and structural phenotypes determined in years 2 and 3.

1997 ARO Biosciences Workshop

POSTER TITLE: "Identification of Mutational Lesions in a Pluriphenotypic Transcriptase Mutant"

Judith A. Lesnaw, Steven A. Enkemann, Michael McGuinness
School of Biological Sciences, University of Kentucky, Lexington KY 40506

Transcription of the genomes of negative-strand RNA viruses is mediated by a viral encoded and multifunctional RNA-dependent transcriptase. Genetic and biochemical studies of the prototype vesicular stomatitis virus (VSV) revealed that RNA-dependent RNA polymerase, guanylyltransferase, guanine-7-methyltransferase, nucleoside-2'-O-methyltransferase, and serine/threonine kinase activities are associated with the large (2109 amino acid) viral L protein. The topological arrangement of these functional domains, and the way in which they interact physically and functionally during RNA synthesis is the focus of our studies.

Ts mutants of VSV, generated *in vivo*, have traditionally served as windows to the complex structure/function correlates within the viral transcription complex. We previously characterized aberrant transcription phenotypes in ts mutants of VSV that map to the L gene by genetic complementation. The mutant F1 displayed a complex phenotype that included ts polymerase activity, and constitutive alterations in methyltransferase activity. A revertant of mutant F1 displayed wild type polymerase activity, but retained differentially altered methyltransferase activity. We proposed that these multiple phenotypic lesions reflect pleiotrophic effects of mutations that alter functional interactions within the transcription complex. In order to correlate the functional alterations with the mutational lesions, we analyzed the sequence of the L genes of ts F1 and its revertant by RT/PCR using the genomes as template. The nucleotide sequence of the F1 L gene differed from that of the corresponding wild type L gene at 13 positions. The nucleotide sequence of the revertant L gene differed from that of the wild type L gene at 16 positions. Comparison of the F1 and revertant L genes revealed only three differences, and these altered codons will be most instructive in determining the structure/function correlates. The locations of the mutations, and a discussion of their potential functional significance will be presented.

AMERICAN SOCIETY FOR VIROLOGY - 13th ANNUAL MEETING
University of Wisconsin-Madison 9-13 July 1994

All abstracts, regardless of country of origin, must be received by February 1, 1994

Name of Presenting Author: Elizabeth Marek
Position: Senior Research Associate
Full Address (Include Dept., Institution, Street, P.O. Box): Department of Biological Sciences, University of Kentucky, 800 Rose Street, 118 Combs Building
City: Lexington State: KY Zip Code: 40536-0084 Country: U.S.A.
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Georgia State University
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Reconstitution of active transcription complexes of Vesicular Stomatatis Virus (VSV) using recombinant L and P proteins expressed in insect cells.

E. Marek, S.A. Enkemann, M. McGuiness and J.A.Lesnaw

Department of Biological Sciences, University of Kentucky, Lexington, KY 40506

Rhabdoviruses have RNA genomes of negative sense RNA. The genome itself is not infectious. Rather for Rhabdoviruses like VSV the infectious agent is a transcription complex that consists of the RNA genome tightly complexed with a nucleocapsid protein (N) and two viral proteins (L and P) that comprise the transcriptase. This transcription complex displays the complete spectrum of enzymatic activities required for the production of functional capped and polyadenylated mRNAs. The catalytic activities are presumed to reside on the L protein but the functional domains have not been mapped. The application of site-directed mutagenesis towards the analysis of the L protein required the production of functional recombinant L protein, and its incorporation into a reconstituted transcription complex. Recombinant Baculoviruses that contain either the VSV L gene (Indiana serotype) or VSV P gene (Indiana serotype) have been constructed. Upon infection of insect cell lines such as Sf9 or High 5™ these recombinant viruses directed the synthesis of large amounts of VSV proteins that comigrated with authentic protein and were recognized by antisera. The recombinant L and P proteins specifically associated with the VSV-derived template (genome + N protein) to form recombinant transcription complexes that produced VSV transcripts in *in vitro* reactions, and initiated a productive infection when transfected into their laboratory host cells.

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ASV Full Member's Name: Judith A. Lesnaw (Print) Full Member's Signature: _____ (Required)

AMERICAN SOCIETY FOR VIROLOGY - 13th ANNUAL MEETING
University of Wisconsin-Madison 9-13 July 1994

All abstracts, regardless of country of origin, must be received by February 1, 1994

Name of Presenting Author: Steven A. Enkemann
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Program Chairman, ASV
Department of Biology
Georgia State University
P.O. Box 4010
Atlanta, GA 30302-4010

This abstract will be used for camera-ready reproduction.

Analysis of L gene mutations using reconstituted transcription complexes of VSV.

S.A. Enkemann, E. Marek, M. McGuiness and J.A.Lesnaw

Department of Biological Sciences, University of Kentucky, Lexington, KY 40506

The transcription complex of VSV directs the synthesis of functional viral mRNAs. These mRNAs are the result of five coordinated enzymatic activities: RNA-dependent RNA polymerase activity, poly A polymerase activity, guanylyltransferase activity, guanine-7-methyltransferase activity, and nucleoside-2'-O-methyltransferase activity. Genetic and biochemical evidence suggest that these activities lie on the large (2109 aas.) L protein. In addition the L protein is believed to have serine/threonine protein kinase activity. The location of these catalytic domains within the L protein is being studied. Sequence comparison among 15 different related L genes revealed dramatic sequence conservation in 17 regions of the L protein. Site-directed mutations were introduced into the cloned L gene to alter conserved amino acids within several of these region. Recombinant Baculoviruses containing the mutated L genes were constructed and used to produce the altered L proteins in infected insect cells. Transcription complexes have been reconstituted with the altered L proteins, recombinant P protein, and virion-derived ribonucleoprotein template. Analysis of these recombinant TCs revealed that alterations in two different regions within the first 750 aas. of the L protein abolish polymerase activity and infectivity. By contrast, alterations in several regions along the C-terminal 500 aas. produced L proteins with reduced but detectable levels of polymerase activity and infectivity.

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LB15 SYNTHESIS AND PROCESSING OF RNA

ALTERATIONS IN TWO DOMAINS, IDENTIFIED AS POSSIBLE REMNANTS OF AN ANCESTRAL RNA POLYMERASE, ABOLISH THE POLYMERASE ACTIVITY OF THE MULTIFUNCTIONAL L PROTEIN OF THE VESICULAR STOMATITUS VIRUS (VSV). S.A. Enkemann, E. Marek, M. McGuinness and J.A. Lesnaw University of Kentucky, Lexington, Ky. 40506.

Synthesis of VSV mRNAs requires five coordinated enzymatic activities: RNA-dependent RNA polymerase activity, poly A polymerase activity, guanylyltransferase activity, guanine-7-methyltransferase activity, and nucleoside-2'-O-methyltransferase activity. The process is directed by a packaged, viral encoded transcription complex (TC) that can be isolated from virions for *in vitro* analysis. The TC consists of the viral genome (negative stranded RNA) complexed with a viral nucleoprotein (N) to form the template, and a two subunit (L and P) transcriptase. On the basis of genetic, biochemical, and sequence analyses, the L subunit (2109 aas.) is postulated to contain the enzymatic activities. Comparison of the amino acid sequences of 15 different related L proteins revealed dramatic sequence conservation in 17 regions. Two of these regions have striking similarity to conserved regions of other RNA polymerases of viral and cellular origin. Conserved amino acids within these regions were altered by site-directed mutagenesis of the cloned L gene. The altered L genes were expressed in insect cells using recombinant baculovirus vectors. Transcription complexes reconstituted with the altered L proteins, recombinant P protein, and virion-derived ribonucleoprotein template exhibited no detectable polymerase activity *in vitro* and failed to initiate a productive infection when transfected into cells. This work was supported by Grant RO1 AI13574 from the National Institute of Health.